COMPARISON OF METHODS FOR DETERMINING NICOTINE AND ITS METABOLITES IN URINE

Gary D. Byrd, Ph.D., and Riley A. Davis, M.S., Res. & Dev., R. J. Reynolds Tobacco Co., and Eeva Kazemi Vala, B.Sc., Svenska Tobaks AB RESERCA

Poster #9
Conference of the Society for Research on Nicotine and Tobacco
San Diego, CA
March 24-25, 1995

Source: https://www.industrydocuments.ucsf.edu/docs/njxk0001

INTRODUCTION

The determination of nicotine and its metabolites in biological fluids plays an important role in understanding nicotine absorption and disposition in humans. Studies have shown that nicotine absorbed by smokers is primarily excreted as phase I (functionalization changes to the xenobiotic) and phase II (conjugation of the xenobiotic with an endogenous compound) metabolites and as unmetabolized nicotine in the urine [1-4]. A proposed metabolic pathway for nicotine in humans is shown in Scheme I. The major products found in human urine are unmetabolized nicotine, cotinine, *trans*-3'-hydroxycotinine, and their glucuronide conjugates. Several minor metabolites have also been reported, including some not shown in this scheme [2].

Various methods for the determination of urinary nicotine and its metabolites have been reported in recent years [4]. Due to interindividual metabolic variability, methods that monitor nicotine and several of its metabolites offer a superior means of assessing nicotine exposure than any assay that determines only one metabolite. In this study, several methods were compared with regard to determination of the major and several minor metabolites and how well they accounted for all forms of nicotine in a standard smokers' urine sample [5].

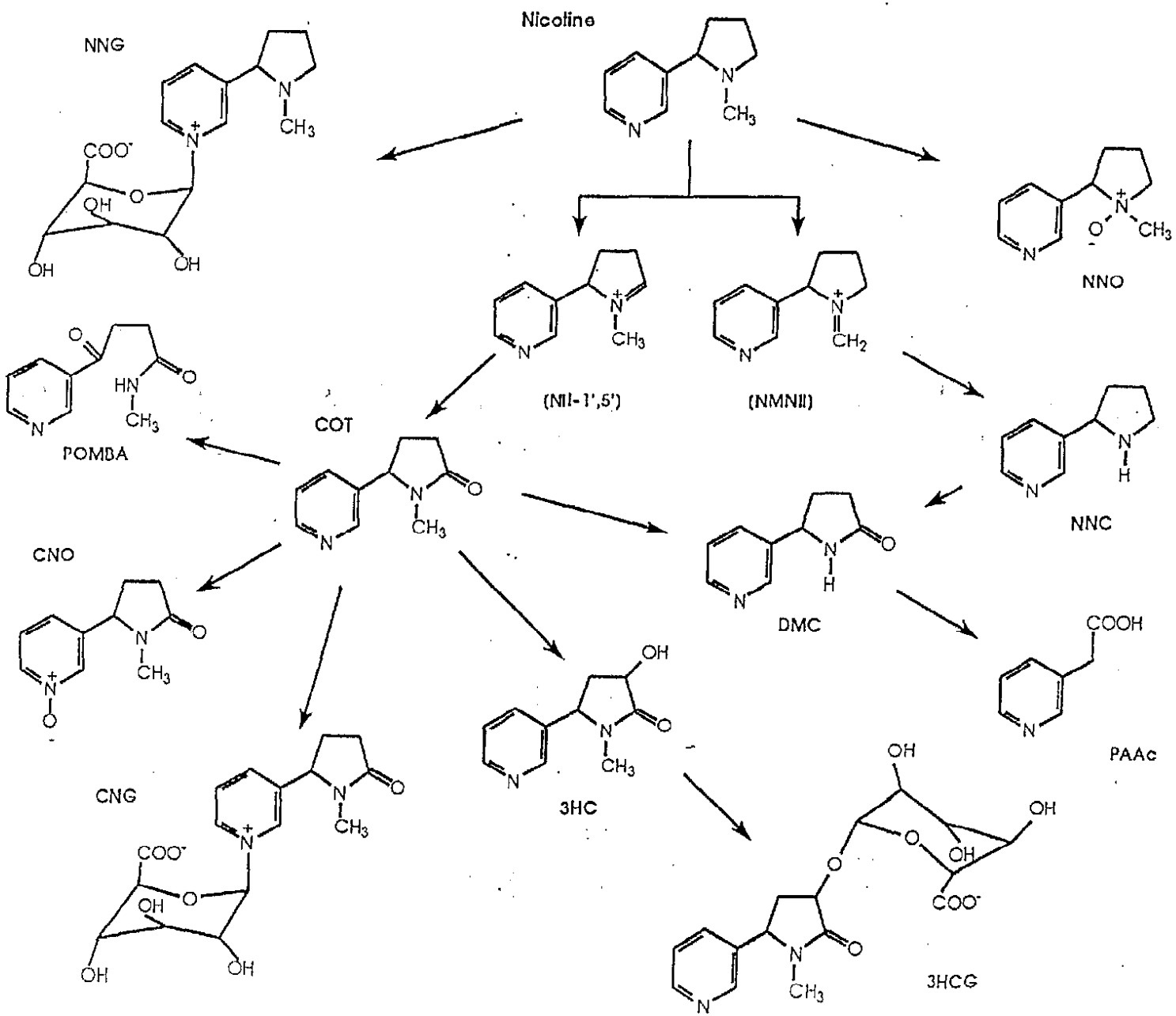
Standards are helpful and often required materials for method development and evaluation. The standard smokers' urine in this study was carefully prepared to be as homogeneous and representative as possbile of smokers urine. Urines collected over 24 h from 9 smokers of ultralow "tar" cigarettes were pooled. The pooled urine was divided into 5-mL aliquots and frozen. Random samples were monitored for creatinine, nicotine, and major metabolites to assess homogeneity. The study included 5 analytical methods and involved 3 different laboratories:

Laboratory	Method
R. J. Reynolds Tobacco Co. (RJR), USA	LC/MS, GC/MS, RIA
Svenska Tobaks AB RESERCA (RES), Sweden	GC/NPD
A general contract research institute (GRI) INBIFO	HPLC, GC/MS

Each laboratory employing instrumental techniques was able to determine phase II metabolites (glucuronides) by monitoring aglycon release following sample hydrolysis. In addition to comparing amounts of individual metabolites, the quantity of total urinary nicotine equivalents was compared.

Source: https://www.industrydocuments.ucsf.edu/docs/njxk0001

NMNII = N-methylnicotine iminium ion, COT = cotinine, DMC = demethylcotinine, NNC = nornicotine, CNO = cotinine-N-oxide, 3HC = trans-3'-hydroxycotinine, COTG = cotinine-N-glucuronide, 3HCG = trans-3'-hydroxycotinine glucruonide, POMBA = 4-(3-pyridyl)-4-oxo-N-methyl-butyramide, PAAc = 3-pyridylacetic acid.



PM3001117513

Source: https://www.industrydocuments.ucsf.edu/docs/njxk0001

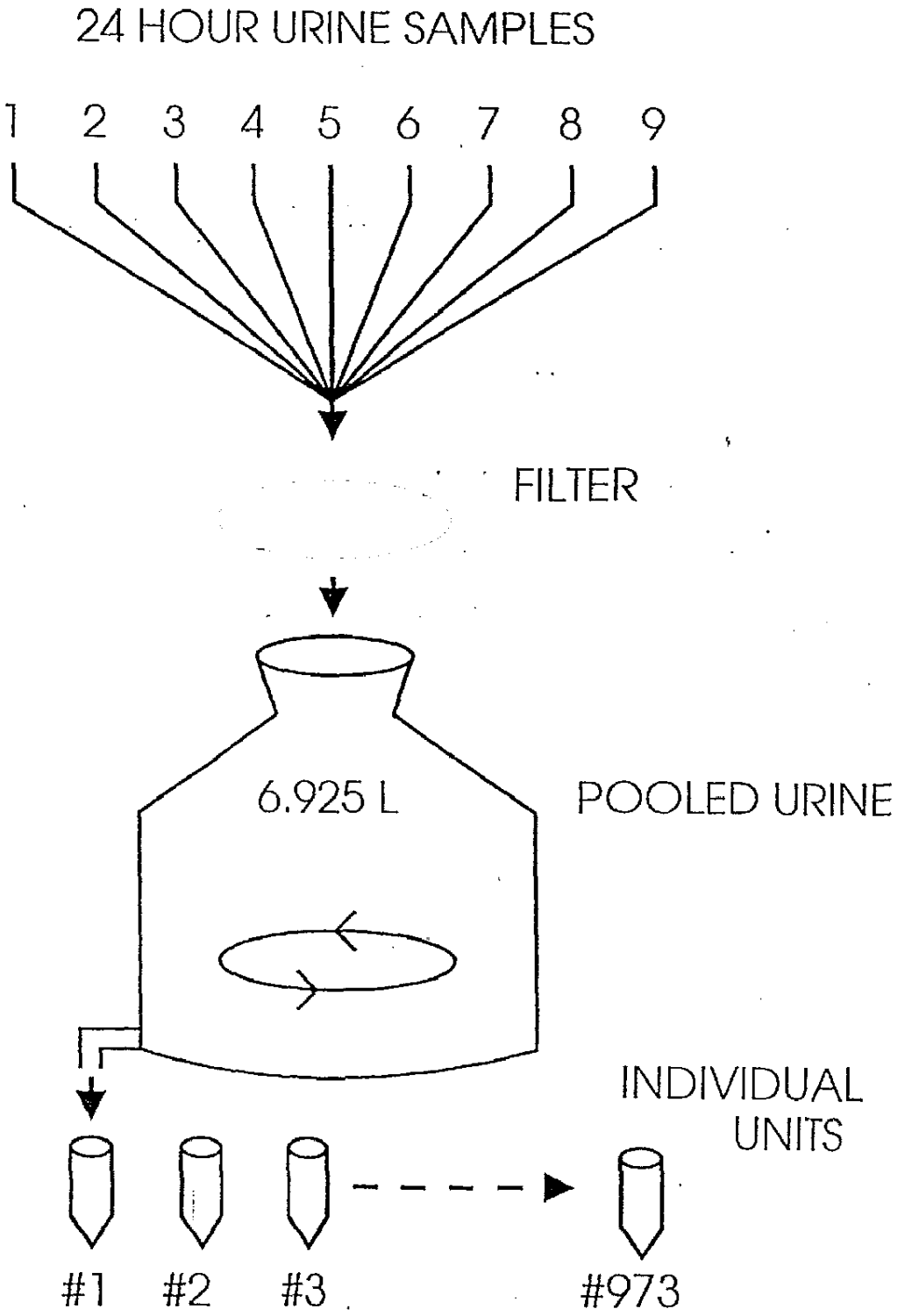
PREPARATION OF STANDARD SMOKERS' URINE

Urine samples were prepared as shown in Scheme II. Twenty-four hour urine samples from 9 male smokers of commercial high end ultralow "tar" cigarettes (5.8 ± 1.7 mg of "tar" and 0.54 ± 0.1 mg of nicotine per cigarette) were pooled, filtered, mixed, and subdivided into 5-mL aliquots. The subjects averaged smoking between 19 and 93 cigarettes per day with an average total nicotine uptake of 21.8 ± 9.0 mg/day based on excreted nicotine and metabolites as determined by the RJR LC/MS method. Each sample was passed through a 19-cm coarse paper filter using vacuum filtration and combined in a large plastic carboy. The final combined volume of the pooled urine was 6.925 L. An automatic pipettor was connected to the carboy and adjusted to dispense approximately 5 mL by drawing from the bottom of the container. The pooled urine sample was constantly stirred and aliquots were collected in 15-mL plastic tubes with screw top caps. Several initial aliquots were dispensed without collection in order to insure system equilibration. Collection was stopped before the remaining volume dropped to less than one liter in order to avoid inhomogeneity effects that might occur at the end of the process. The tubes were numbered in the order collected and the final number of tubes was 973. Each tube was capped tightly and immediately placed in a freezer at -20°C.

Homogeneity of the samples was assessed by comparing concentrations of creatinine, nicotine, cotinine, and trans-3'-hydroxycotinine across 12 samples drawn at random.

Source: https://www.industrydocuments.ucsf.edu/docs/njxk0001

SCHEME II Preparation of Standard Smokers' Urine.



PM3001117515

Source: https://www.industrydocuments.ucsf.edu/docs/njxk0001

METHODS

All samples were selected at random for analysis. Methods used by the respective laboratories are listed below:

RJR: LC/MS analyses were performed according to a published method [6,7]. GC/MS analyses were performed using a modification [5] to a published method [8]. RIA analyses were performed using polyclonal antibodies according to published methods [9-11]. ELISA analyses were performed using monoclonal antibodies according to a published method [12].

RES: GC/NPD analyses were performed according to a published method [13].

GRI: HPLC analyses following derivatization with 1,3-diethyl-2-thiobarbituric acid were based on a published method [14]. GC/MS analyses were performed based on a published method [8].

Source: https://www.industrydocuments.ucsf.edu/docs/njxk0001

Data Summary for Standard Smokers' Urine - Individual Metabolites (nmol/mL)

Lab (Method)	NIC	NNG	COT	CNG	t3HC	t3HCG	NNO	CNO	DMC	POMBA	PAAc	NNIC	c3HC
GRI (HPLC)								*					
Avg (n=5)	11.67	3.33	8.24	15.06	23.23	9.38	6.29	2.24	1.98	1.25	0.22	0.61	0.21
StDev	0.31	0.56	0.23	0.80	0.68	0.52	0.11	0.47	0.06	0.10	0.00	0.07	0.00
GRI (GC/MS)													
Avg (n=5)	11.79	**	9.20	**	24.22	**	**	**	1.17	0.63		0.54	0.36
StDev	0.31		0.17		0.47				0.00	0.00		0.07	0.05
RES (GC/NPD)													
Avg (n=5)	9.11	2.93	8.09	11.95	23.07	11.58	8.90	1.30					
StDev	0.80	0.41	1.05	1.22	1.74	1.56	0.85	0.11					
RJR (LC/MS)													
Avg (n=10)	12.58	5.06	9.03	15.02	20.96	5.79	6.39	2.87	0.89				
StDev	1.76	1.42	0.61	1.42	1.79	1.09	0.46	0.15	0.22				
RJR (GC/MS)													
Avg (n=10)	16.30 ***		23.10 ***		32.70 ***								
StDev	0.60		1.06		4.67								
RJR (RIA)													
Avg (n=2)	14.14 ****		23.07 ****										
StDev	2.04		5.97										
RJR (ELISA)													
Avg (n=2)	14.26 ****		19.49 ****										
StDev	2.41		2.33										
AVERAGE	11.29	3.77	8.64	14.01	22.87	8.91	7.20	2.14	1.35	0.94	0.22	0.57	0.29
StDev	1.51	1.13	0.56	1.78	1.37	2.92	1.48	0.79	0.56	-	-	-	-
n =	4	3	4	3	4	3	3	3	3	2	1	2	2

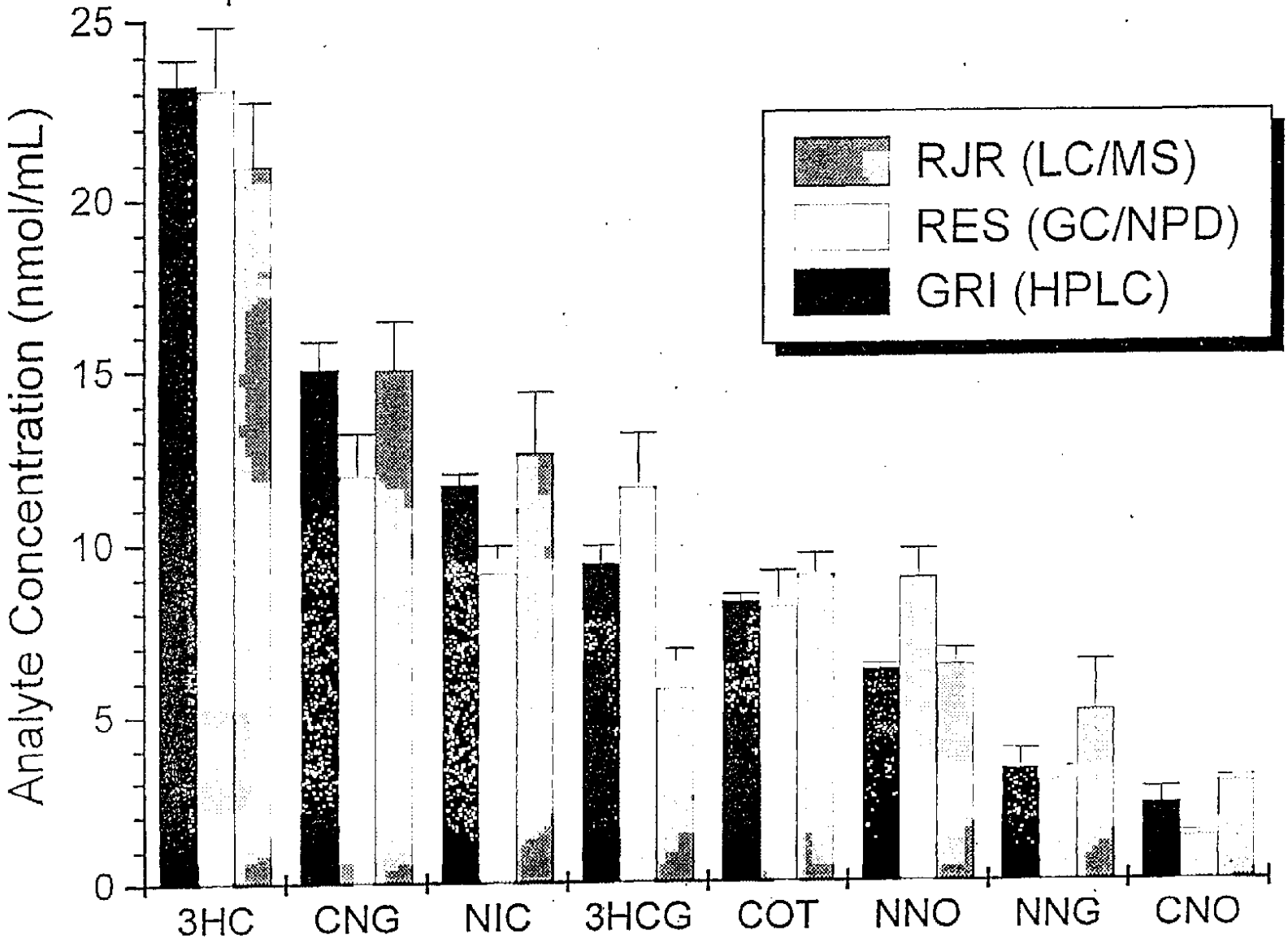
*Determination after TiCl3 reduction.
**Determination by GC/MS not feasible at time of analysis.
***Analysis on hydrolyzed urine (data include free and conjugated forms); not averaged with indiv. metabolites.
****Cross-reactivity from conjugates and other metabolites unknown; not averaged with individual metabolites.

PM3001117517

Source: https://www.industrydocuments.ucsf.edu/docs/njxk0001

Data Summary for Standard Smokers' Urine

Comparison of individual metabolite data from most comprehensive methods.



PM3001117518

Source: https://www.industrydocuments.ucsf.edu/docs/njxk0001

(Most comprehensive methods only)

Lab (Method)	TOTAL NICOTINE
GRI (HPLC)	83.69
Avg (n=5)	
RES (GC/NPD)	76.93
Avg (n=5)	
RJR (LC/MS)	78.59
Avg (n=10)	
RJR (GC/MS)	72.09
Avg (n=10)	
AVERAGE	**77.83**
StDev	4.79
n =	4
Rel StDev	6.15%

PM3001117519

Source: https://www.industrydocuments.ucsf.edu/docs/njxk0001

RESULTS

- Homogeneity assessment of the Standard Smokers' Urine showed no correlation with sample number and the concentration determined for creatinine, nicotine, cotinine, and *trans*-3'-hydroxycotinine.

- Four of the methods - GRI's HPLC, RES's GC/NPD, RJR's LC/MS, and RJR's GC/MS - were "comprehensive" in that they determined all the major metabolic forms of nicotine found in human urine.

- The four comprehensive methods showed good agreement in accounting for total nicotine (relative standard deviation < 7%).

- The methods showed good agreement among measured concentrations of all major metabolites (relative standard deviation < 30%) except for *trans*-3'-hydroxycotinine.

- The immunoassay methods, though not comprehensive in scope, accounted for approximately half the amount of nicotine and metabolites as the comprehensive methods.

- The RIA and ELISA data for nicotine are the same; however, the RIA yielded cotinine values approximately 15% higher than the ELISA. This difference may represent total cross-reactivity of the glucuronide.

CONCLUSIONS

- A Standard Smokers' Urine sample is a valuable tool in qualifying methods for the determination of nicotine and its metabolites in urine.

- More comprehensive methods show good agreement in accounting for total urinary nicotine and its metabolites.

ACKNOWLEDGEMENTS

I would like to thank M. E. Lovette (RJR) for preparation of the standard smokers' urine and M. J. Morton (RJR) for statistical assistance.

Source: https://www.industrydocuments.ucsf.edu/docs/njxk0001

1. N. L. Benowitz. In The Pharmacology of Nicotine, M. J. Rand and K. Thurau, eds., IRL Press, Oxford, Washington, pp. 3-18 (1988).

2. G. A. Kyerematen and E. S. Vessell. Drug Metabolism Reviews, 23, 3-41 (1991).

3. M. Curvall, E. Kazemi Vala, and G. Englund. In *Effects of Nicotine on Biological Systems* (Adlkofer, F., and Thuraus, K., Eds.) pp. 69-75, Birkhäuser Verlag, Basel (1991).

4. G. D. Byrd, J. H. Robinson, W. S. Caldwell, and J. D. deBethizy. Rec. Adv. Tob. Sci., 20, 5-31 (1994).

5. G. D. Byrd. 41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, May 31 - June 4, 1993.

6. K. T. McManus, J. D. deBethizy, D. A Garteiz, G. A. Kyerematen, and E. S. Vesell. J. Chrom. Sci., 28, 510-516 (1990).

7. G. D. Byrd, K.-M. Chang, J. M. Greene, and J. D. deBethizy. Drug Metabolism and Disposition, 20, 192-197 (1992).

8. P. Voncken, G. Schepers, and K.-H. Schafer. J. Chromatogr., 479, 410-418 (1989).

9. J. J. Langone, H. B. Gjika, and H. Van Vunakis. Biochemistry, 12, 5025-5030 (1973).

10. J. J. Langone and H. Van Vunakis. In Methods in Enzymology, J. J. Langone and H. Van Vunakis, eds., Academic Press, New York, vol. 84, 628-640 (1982).

11. H. Van Vunakis and J. J. Langone. In Environmental Carcinogens Methods of Analysis and Exposure Measurement,, I. K. O'Neil, K. D. Brunnemann, B. Dodet, and D. Hoffman, eds., W. H. O. International Agency for Research on Cancer, Lyon, 317-330 (1987).

12. J. L. Langone, G. Cook, R. J. Bjercke, and M. H. Lifschitz. J. Immunol. Meth., 114, 73-78 (1988).

13. G. Andersson, G. Bjornberg, and M. Curvall. J. Oral Pathol. Med., 23, 161-167 (1994).

14. K. Rustemeier, D. Demetrios, G. Schepers, and P. Voncken. J. Chromatogr.: Biomed. Appl., 613, 95-103 (1993).

Source: https://www.industrydocuments.ucsf.edu/docs/njxk0001